Journal of Applied Botany and Food Quality 86, 190 - 197 (2013), DOI:10.5073/JABFQ.2013.086.026

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Interaction of drought stress and UV-B radiation – impact on biomass production and flavonoid metabolism in lettuce (*Lactuca sativa* L.)

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(Received May 30, 2013)

Summary

The response of plants to stress such as UV-radiation or drought highly depends on the species, cultivar, plant organ, developmental stage, and furthermore, is influenced by ecophysiological interactions. Drought stress as well as UV irradiation are the most adverse factors for plant growth and productivity. In the present study, the interactive effect of UV-B and drought stress on biomass, primary and secondary metabolites, and mediated enzyme activity of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) was investigated in lettuce (*Lactuca sativa* L.). It was found that biomass production decreased in response to both stressors, while dry matter, total phenolic content and the flavonol quercetin were not significantly affected by UV-B and drought stress, neither solely nor in combination. In contrast, anthocyanins and luteolin accumulated only in response to drought stress. However, the precursor amino acid proline as well as the activity of PAL increased under conditions of increased UV-B and water deficit. Thus, the present results deduce that both stressors acted either synergistically or to some extent antagonistically in terms of inducing plant protective mechanisms.

Introduction

Plant growth and quality are affected by various environmental impacts. Therefore, acclimatization of plants to changing environmental conditions is essential for their growth and survival. Drought stress is one of the most important environmental stressors, adversely affecting crop productivity and quality. In many habitats, water shortage is the main limiting factor of plant productivity being regarded as a consequence of global climate change (Tesar et al., 2007). Drought stress induces various physical and/or chemical modifications in plants. Liu et al. (2011) reported that the photosynthetic electron chain in plants is affected and thus, photosynthetic pigments decreased in response to water deficit (Ben Ahmed et al., 2009). Drought stress may also alter the synthesis of secondary plant compounds. For example, the content of phenolic compounds such as caffeic acid was found to be reduced in *Ipomoea batatas* roots (Mao et al., 2004), whereas total soluble phenols, quercetin and betulinic acid were increased in *Hypericum brasiliense* (shoots) (Abreu and Mazzafera, 2005) under drought stress conditions. Further, selected amino acids are also involved in drought stress responses: Proline is widely distributed in plants and is accumulated in larger amounts than other amino acids in drought stressed plants (Irigoyen et al., 1992). For various crops, it is reported that the proline content significantly increased when water is limited, e.g. for maize and bean plants (shoots and roots) (Mohammadkhani and Heidari, 2008), and for alfalfa plants (leaves) (Irigoyen et al., 1992). Thus, drought stress mediated changes in primary and secondary plant compounds differ depending on the plant and morphological plant part used for consumption.

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In recent years, increasing UV-B radiation resulting from air pollution-induced ozone depletion has raised awareness of the effects of UV-B on the ecosystem. Though, only a small portion of the total solar spectrum, UV-B in the range of 280-315 nm has a large effect, as it may induce photobiological stress and activates the plant defence system, leading to an accumulation of secondary plant metabolites and corresponding enzymes in plant tissues (Teramura, 2006). Many studies report potential consequences of UV-B radiation on plants (e.g. Jansen, 2010), but there is still a rather limited understanding of the effect on secondary plant metabolites. Secondary plant metabolites such as phenolic compounds (flavonoids, hydroxycinnamic acids) act often as UV-B absorbing components or as natural antioxidants in plants (Treutter, 2010). The protective health effects of flavonoids are attributed to their radical scavenging and metal-chelating abilities and can be ascribed to their capacity to transfer electrons as free radicals, chelate heavy metals, activate antioxidant enzymes, reduce α-tocopherol radicals and/or inhibit oxidases (Heimler et al., 2007). Thus, UV light may act as a natural elicitor of secondary metabolite responses in higher plants.

Recently, the targeted application of UV irradiation has gained importance among food scientists. Main targets are sanitation purposes and prevention of postharvest diseases (Terry and Joyce, 2004) as well as improving the functionality of fruits and vegetables, i.e. health-promoting properties of plant food (Schreiner and Huyskens-Keil, 2006; Schreiner et al., 2012). The general emerging elicitor effects of low UV-B radiation, triggering distinct changes, e.g. in the accumulation of phenolic compounds were reported by Huyskens-Keil et al. (2007), Treutter (2010) and Schreiner et al. (2012). This was specifically documented for quercetin in onion bulbs and anthocyanins in strawberry fruits (Higashio et al., 2005) as well as for diverse flavonoids in brassica sprouts (Huyskens-Keil et al., 2008) and black currant fruits (Huyskens-Keil et al., 2007).

UV-B as well as drought are known to promote the activity of enzymes playing an important role in phenol metabolism, such as phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) (Dixon and Paiva, 1995; Treutter, 2010). Stress mediated PAL synthesis induced by UV-B has been documented for lettuce (Caldwell and Britz, 2006) and white asparagus spears (Eichholz et al., 2012). The promotion of PAL activity through water deficit conditions was reported for pepper plants (Sung et al., 2005). Further, plant responses to UV-B are known to interact with the water availability of plants, i.e. in general, water limitation is reported to lower the UV-B sensitivity of plants (Gwynn-Jones et al., 1999). However, the sensitivity of plants to UV-B or drought differs among species, populations and varieties, and depends upon physiological stage of the plant and duration of stress impact (Abreu and Mazzafera, 2005; Schreiner et al., 2009; Liu et al., 2011). Information on changes of the secondary plant compounds as affected by drought stress in combination with UV-B radiation is contradictive to a certain extent and still limited (Hofmann et al., 2003).

For the present study, lettuce (*Lactuca sativa* L.) was used as a model

plant, as it is one of the most important dietary leafy vegetables that is primarily consumed fresh or consumed as fresh-cut convenience product (PUTNAM et al., 2000). Lettuce reveals high contents of health-promoting compounds such as phenolic components (gallic acid, caffeic acid), carotenoids (lutein), and dietary fiber (CHU et al., 2002; CALDWELL, 2003; NICOLLE et al., 2004). Moreover, it is reported that lettuce indicates plant protective mechanisms towards stress impacts, e.g. drought stress or UV-B irradiation, by activation of genes being responsible for PAL biosynthesis (OH et al., 2010) or by triggering the synthesis of anthocyanins and other flavonoids, respectively (TSORMPATSIDIS et al., 2010).

Consequently, the aim of the present study was to investigate the interactive effect of drought stress and UV-B radiation on the plant defense reaction of lettuce analyzing the amino acid proline as a stress indicator as well as the profile of flavonoids with the corresponding PAL activity. This study might contribute to an assessment of multiple moderate stress factors on the dynamics of health promoting plant metabolites in vegetables as demonstrated for lettuce.

Material and methods

Plant Material, Water Regime and UV-B Treatment

Seeds of *Lactuca sativa* var. *capitata* cv. Teodore RZ® were obtained from Rijk Zwaan Ltd., Netherlands. All plants were grown for two months (April-May 2011) in a greenhouse located at the experimental station of the Humboldt-Universität zu Berlin in Berlin-Dahlem, Germany. A completely randomized block design was used for experimental purposes. Two weeks before harvest, plants were divided into two groups. The first group (well-watered: WW) and the second group (drought stress: DS) were grown in a peat substrate with 45% and 25% water capacity, respectively. The different water capacities were determined daily and obtained gravimetrically. One week before harvest, each group (WW and DS treatment, each n=30 plants) was again divided into sub-groups, i.e. well-watered without UV-B (***control***), well-watered with UV-B (***UVB***), water-deficit without UV-B (***DS***), water-deficit with UV-B (***DS+UVB***). The two sub-groups ***UVB*** and ***DS+UVB*** (each n=15 plants) were subjected to UV-B radiation at 0.11 kJ m^{-2} for 5 days using an UV-B fluorescence light source (FL-20SE, 305-310 nm, Philips GmbH, Germany) with an average fluency rate of 8.2 Ws m^{-2} at a mean distance of 20 cm to plants. The other two sub-groups were used as control. Selected water conditions and UV-B treatments have been evaluated in preliminary experiments.

After harvest, all plants were weighted in order to determine the total above-ground biomass. Afterwards, all infected or damaged leaves were removed and plants were weighted again to obtain the marketable yield that is presented as biomass in this study. During the experiment, the following parameters were determined: dry matter, proline content, total phenolic content, flavonoid profile, and PAL activity.

Chemical analysis

For the chemical analysis, harvested plants (n=15 per treatment) were shock-frozen in liquid nitrogen and stored at -25 °C. For phenolic compound analysis (total phenolic content, flavonoids) and the determination of proline, samples were lyophilized (ALPHA 1-4. Christ, Osterode am Harz, Germany), ground and stored in desiccators until further analysis. Additionally, for determination of chlorophyll and carotenoid contents as well as PAL activity, frozen samples were stored at -80 °C until further analysis.

Analysis of chlorophyll and carotenoids

Frozen samples (500 mg fresh weight) were homogenized in 15 mL aceton: hexan (4/5, v/v). The homogenate was centrifuged at 4000 rpm for 20 min (Labofuge, Heraeus, USA). In the hexan layer of the supernatant the contents of total carotenoids, β-carotene, lutein, lycopene, chlorophyll a and chlorophyll b were assayed spectrophotochemically as described by GOODWIN (1980). The absorbance of the extract was measured at 445 nm, 450 nm, 453 nm, 505 nm, 645 nm and 663 nm, respectively. Pigment contents were expressed as micrograms or milligrams per gram dry matter (μg g^{-1} or mg g^{-1} DM).

Extraction of phenolic compounds and analysis of total phenolic content

For the analysis of total phenolic content, extraction was conducted according to CONNOR et al. (2002) using acidified methanol (0.1% hydrochloric acid). An aliquot of 0.5 g freeze-dried sample was mixed with 3 mL of acidified methanol and centrifuged for 15 min at 4000 rpm and repeated three times. Supernatants were collected and standardized to a final volume of 10 mL. Total phenolic content of the leave's extract was determined using the Folin-Ciocalteu methodology (SLINKARD and SINGLETON, 1977). Absorbance was measured photometrically after one hour incubation time at a wavelength of 765 nm (LKB-Novaspek II, Pharmacia, Freiburg, Germany). Results were expressed as milligrams gallic acid equivalents (GAE) per gram dry matter (mg GAE g^{-1} DM).

HPLC analysis of selected flavonoids

Lyophilized samples (0.2 g) were extracted with 10 mL of acidified methanol (0.1% HCl) and centrifuged at 1210 × g for 10 min. The supernatants were evaporated in a water bath at 50 °C and 200-300 mbar in a rotator evaporator. Then, dried samples were resolved with 2 mL of 2 N HCl and 2 mL of methanol (HPLC grade) and heated in water bath at 80 °C for 2 hours. The volume was set to 5 mL with methanol (HPLC grade). These extracts were kept at -20 °C for HPLC analysis.

An analytical Hewlett Packard 1100 series HPLC instrument equipped with an autosampler, quarternary HPLC pump and diode array detector was used. Analytical separation of the flavonoids was carried out on a 150 × 3 mm, Prodigy OD 53 column (Phenomenex Aschaffenburg, Germany) with a two solvent mobile phase (eluent A = water/acetic acid/acetonitrile (98.5/0.5/1; v/v/v); eluent B = acetonitrile). The eluent gradient used for all extracts was described as follows: 0% B (5 min); 0-4% B (4 min); 4% B (6 min); 4-8% B (15 min); 8-22% B (15 min); 22-28% B (5 min); 28% B (5 min); 28-45% B (10 min), and 45-0% (1 min). The detection was performed at 280 nm, 325 nm and 365 nm, simultaneously. The injection volume was 20 μL, the flow rate was 0.5 mL min^{-1} and the column temperature was 30 °C. Total concentration of quercetin and luteolin was obtained from a 1 mM standard solution. Results were expressed as milligrams per gram dry matter (mg g^{-1} DM).

Anthocyanin analysis

For anthocyanin analysis, the absorbance of a methanolic extract, similarly prepared for the HPLC analyses, was measured spectrophotometrically (Heraeus, USA) at a wavelength of 510 nm. The anthocyanin content was calculated by using the molar extinction coefficient of 29,600 L cm^{-1} mol^{-1} and the molecular weight of 449.2 g mol^{-1} of cyanidin-3-glucoside. The anthocyanin content was calculated on the base of dry matter as milligrams cyanidin-3-glucoside per gram dry matter (mg Cy g^{-1} DM).

PAL activity assay

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity was determined as described by ZUCKER (1968) with slight modifications.

An aliquot of 6 g of fresh lettuce tissue was homogenized with 50 mL 0.025M borate buffer (pH 8.8) containing 5 mM of 2-mercaptoethanol. The homogenate was filtrated and centrifuged at 11000 rpm at 4 °C for 15 min. The supernatant obtained was used as a crude extract for PAL analysis. The reaction solution consisted of 1.5 mL of a 0.025 M borate buffer (pH 8.8), 0.5 mL of 0.1 M L-phenylalanine and 0.3 mL of the crude enzyme solution. The PAL activity was measured after an incubation period of 60 min at 37 °C photometrically (Heraeus, USA) at 290 nm to determine the increase in cinnamic acid as a product. Protein concentration of the extract was determined according to BRADFORD (1976) method using BSA as standard. The enzyme activity was expressed as picokatal per milligram protein (pkat mg^{-1} protein).

Proline assay

Proline content was determined according to BATES et al. (1973) with slight modifications. A freeze-dried sample (40 mg) was mixed with 1.5 mL of 3% aqueous sulfosalicylic acid in reaction tubes. Samples were centrifuged at 0 °C and 11000 rpm for 30 min. To 300 µL of the supernatant 300 µL of ninhydrin and 300 µL of glacial acetic acid were added and mixed in a reaction tube for 1 h at 90 °C. Reaction was stopped in an ice bath for 5 min. The mixture was extracted with 900 µL toluene and centrifuged 4000 rpm for 10 min. The absorbance of the toluene layer was determined photometrically at 520 nm. Pure proline (L-proline, 99%, Roth, Germany) was used as a standard. Results were expressed as milligrams per gram dry matter (mg g^{-1} DM).

Statistic calculations

The statistical evaluation was performed using SPSS 13.0 (SPSS Inc., USA). Significance of differences was conducted using ANOVA with a Tukey test ($p \leq 0.05$). The mean variability was indicated by the standard deviation.

Results and discussion

Effect of drought stress and UV-B radiation on biomass production and dry matter content

In the present study, biomass production, i.e. above-ground fresh weight of plants of all treatments revealed a significant loss compared to the control plants (Fig. 1). UV-B treated plants and plants grown under both stress conditions (DS+UV-B) exhibited significantly lower biomass production than plants grown only under drought stress conditions.

The worldwide increase of drought stress conditions is hypothesized to lead to a reduction of biomass production, and thus a reduction of total plant yield (FAROOQ et al., 2009). During water deficiency periods, plants close their stomata to prevent water loss by transpiration which correspondingly permits less CO_2 intake into plants. This is associated with a decline in photosynthetic activity leading to a reduced biomass production (FAROOQ et al., 2009). However, in the present study, photosynthetic pigments were not affected by drought stress conditions (data not shown) indicating a limited effect of drought stress on photosynthesis in lettuce. This is underlined by the results obtained on the dry matter content of lettuce studied. The dry matter content of lettuce grown under water-deficit conditions (DS) was significantly higher compared to UV-B treated plants, but not significantly different to control plants (Fig. 2). In general, it is reported that dry matter content of plants grown under limited water availability increases due to the higher accumulation of assimilates (MARSCHNER, 1995) that are necessary for maintenance of plant metabolism or activation of stress responses (ROITSCH, 1999). Carbohydrates might accumulate under drought stress conditions, e.g. sucrose and sugar alcohols (SIRCELJ et al., 2005), being osmoprotectants protecting plants from extreme oxidative stress (RONTEIN et al., 2002).

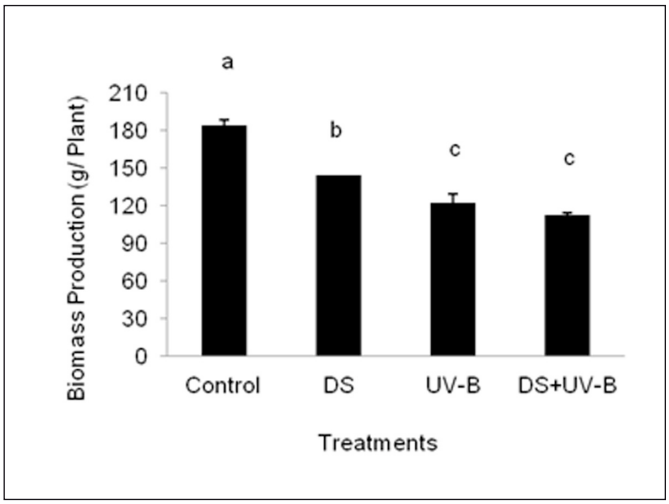

Fig. 1: Biomass production (g $plant^{-1}$) of lettuce plants at harvest in response to drought stress, UV-B radiation and the combination of both stress factors. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

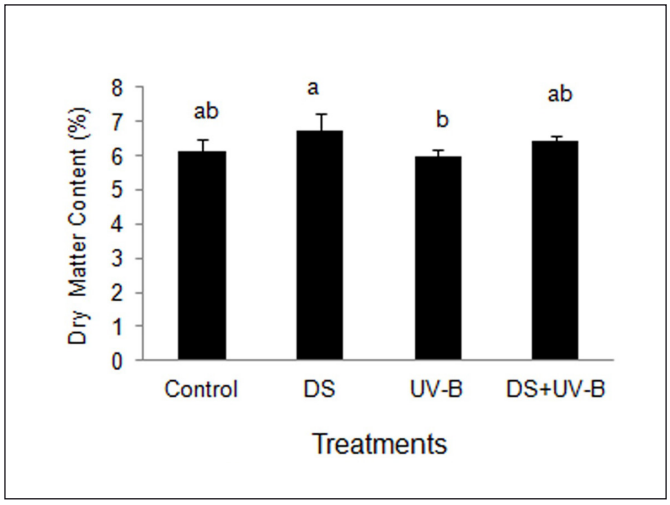

Fig. 2: Dry matter content (%) of lettuce at harvest in response to drought stress, UV-B radiation and the combination of drought stress and UV-B radiation. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

While crop biomass production was highly sensitive to UV-B radiation (Fig. 1), dry matter content remained constant in response to UV-B (Fig. 2). This data is supported by findings of CECHIN et al. (2007), who reported that inhibition of photosynthesis is a key reaction leading to a decline in biomass production of crops. Plants exposed to UV-B might allocate more energy for other physiological activities than biomass production, especially defense mechanisms

resulting in an UV-B mediated synthesis of protective pigments, such as carotenoids, anthocyanins or phenolic acids (GAO et al., 2004).
Plant response to UV-B is also dependent on other ecophysiological factors (CALDWELL, 2003), some of them even in an antagonistic manner (ALEXIEVA et al., 2001). In the present study, UV-B treatment of drought stressed plants amplified the effect of both stress factors on biomass production compared to drought stress alone. However, additional drought events in UV-B treated plants did not lower dry matter production of lettuce. Thus, UV-B might have a stronger impact on biomass production in lettuce than drought stress.

Effect of drought stress and UV-B radiation on proline content

The amino acid proline is a pre-requisite marker of drought stress (ALEXIEVA et al., 2001) and may also act as a protective factor against UV stress (HE et al., 2011). In the present study, proline increased only tendentiously in response to drought stress compared to control plants, while UV-B treated plants showed no significant effect on the proline content (Fig. 3).
Previous studies documented an accumulation of intracellular proline concentration in response to osmotic stresses, such as drought (SINGH et al., 1973). This amino acid is generally assumed to serve as a physiologically compatible solute that maintains a favorable osmotic potential between cells (POLLARD and WYN, 1979), and is also involved in alleviating cytosolic acidosis in plants under stress conditions (KURKDJIAN and GUERN, 1989). Also, enhanced UV-B radiation has been reported to increase concentrations of free proline (HOFMANN et al., 2003). However, results of the present study did not confirm this finding. This might be due to the UV-B radiation dosage applied, which did not influence proline synthesis at this stage. From the present findings it is assumed that drought conditions revealed a tendentiously higher influence on proline content in lettuce than UV-B radiation.
The combination of UV radiation and drought stress applied to the lettuce plants of the present study revealed a pronounced increase in comparison to the single stress factor: Proline concentrations rose up to 1.5 times higher than in control plants and plants subjected to single UV-B treatment (Fig. 3). When UV-B irradiation and drought stress is applied simultaneously or successively, the specific impact of UV-B on drought is reported to be reduced compared to the single stress factor (OH et al., 2010). Authors assumed that plant response to drought stress might be masked or compensated by other stress factors. The latter supports the present findings, however, the compensated effect of combined stress on proline content was found to occur only tendentiously compared to single drought stress conditions.

Effect of drought stress and UV-B radiation on phenolic compounds

Plant tissue protection against stress is a concerted action of diverse enzymatic and non-enzymatic antioxidant mechanisms. Enzymatic defence includes e.g. peroxidases (POD), glutathione reductase (GR) and phenoloxidases (PPO), while non-enzymatic antioxidant network includes e.g. the synthesis of phenolic compounds (especially flavonoids), carotenoids and ascorbic acid (MANDAL et al., 2009). Thus, phenolic compounds provide important physiological and ecological duties, being mainly involved in protection against different types of stress (AYAZ and LU, 2000).

Total phenolic content

In the present study, single drought or UV-B treatment as well as the combination of both stressors did not lead to changes in total phenolic content of lettuce plants compared to control plants (Fig. 4). This is in contrast to various studies, where one of the main actions of plants in stress situations such as drought or UV-B exposure is the synthesis and accumulation of phenolic compounds. An increase in total phenolic content as a response to drought stress and UV radiation was reported in numerous reports (ABREU and MAZZAFERA, 2005; HUYSKENS-KEIL et al., 2008; TREUTTER, 2010). Polyphenols act against stress in plants, however, dependent on the stress factor different phenol groups or single compounds are synthesized and accumulated (DIXON and PAIVIA, 1995). Thus, changes within the

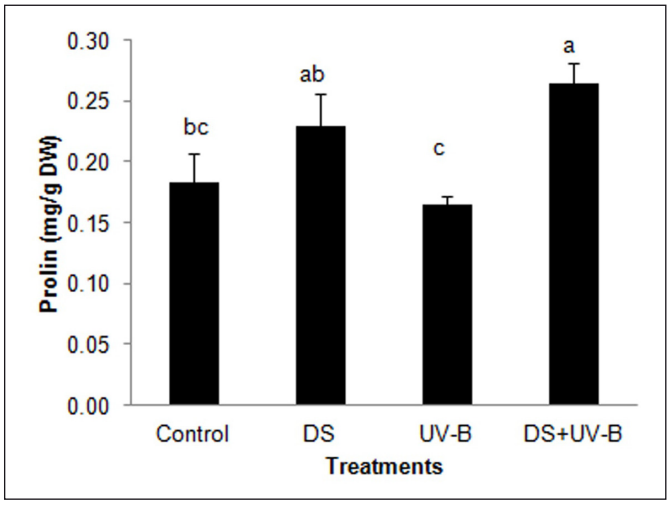

Fig. 3: Proline content (mg g^{-1} DM) of lettuce at harvest in response to drought stress, UV-B radiation and the combination of drought stress and UV-B radiation. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

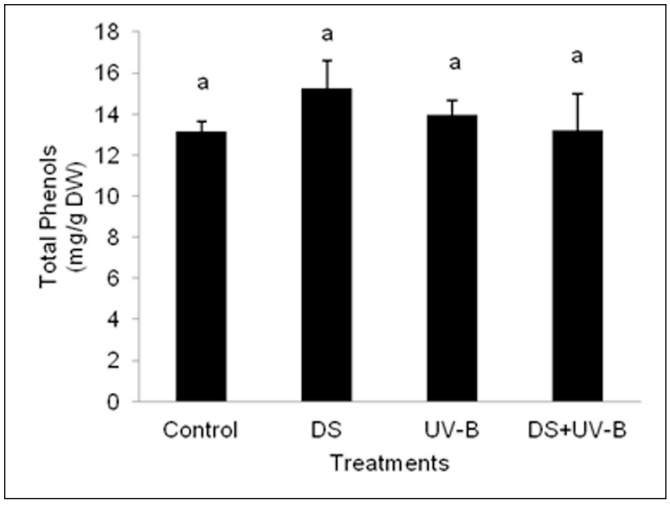

Fig. 4: Total phenolic content (mg GAE g^{-1} DM) of lettuce at harvest in response to drought stress, UV-B radiation and in combination of drought stress and UV-B radiation. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

phenolic profile could occur without influence on the total content of phenolic compounds. Moreover, the assay used for the analysis of the total phenolic content determines the changes of all compounds with reducing abilities as sum parameter (AL-DUAIS et al., 2009). Consequently, when considering the results of the flavonoid profile, the effect of the different treatments on phenolic compounds of lettuce demonstrated a more specific picture.

Flavonoid profile

In this study, the main flavonoids of lettuce detected were quercetin, luteolin and anthocyanin. For quercetin, stress application did not lead to significant changes. However, results revealed a tendentious increase in UV-B treated plants and tendentiously decline of quercetin in lettuce grown under combined stress conditions (DS+UV-B) (Fig. 5). In contrast, analysis of luteolin showed different results (Fig. 6). Here, drought exhibited a significantly promoted synthesis of luteolin compared to control plants. UV-B treated plants and plants grown under both stress factors (DS+UV-B) experienced no significant changes in luteolin concentration. Here, only a tendentious accumulation of luteolin was found.

Drought stress activates genes associated with the protection through polyphenol synthesis, leading to an accumulation of different phenolic compounds, such as chlorogenic acid, chicoric acid, caffeic acid, quercetin, luteolin, and others (MAO et al., 2004; ABREU and MAZZAFERA, 2005). Drought not only influences the water status of plants, but it also leads to oxidative stress (ZHU, 2002). Antioxidants such as phenolic compounds are able to prevent oxidative burst of plant cells and thus, protect plants from damage of proteins, lipids, DNA as well as RNA (APEL and HIRT, 2004). However, this study suggests that in drought stressed plants, luteolin might play a more pronounced role in the protective mechanisms in lettuce than quercetin.

Flavonoids strongly absorb UV and their biosynthesis is known to be accelerated by UV irradiation (SCHREINER et al., 2012). It seems that UV-B stimulates selectively those flavonoids with antioxidant properties. UV-B shielding by flavonols, i.e. increase of quercetin

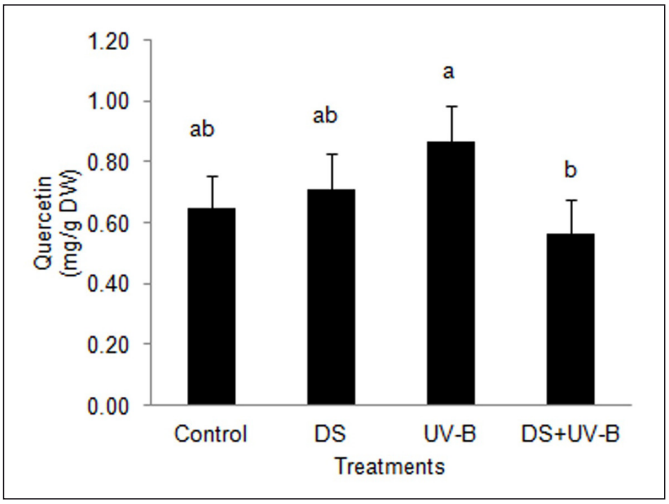

Fig. 5: Quercetin content (mg g^{-1} DM) of lettuce at harvest in response to drought stress, UV-B radiation and in combination of drought stress and UV-B radiation. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

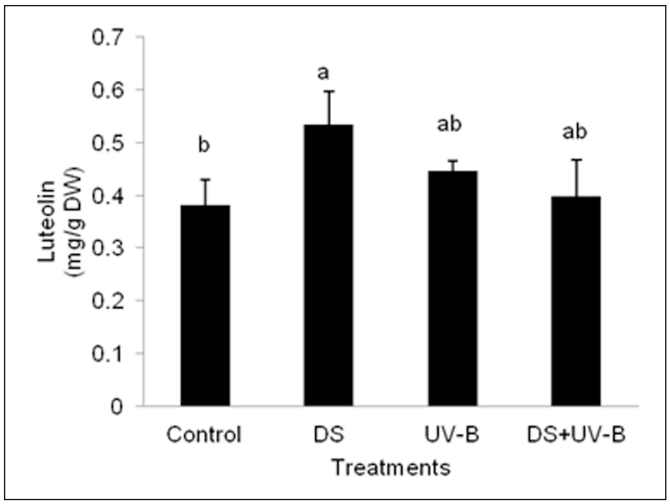

Fig. 6: Luteolin content (mg g^{-1} DM) of lettuce at harvest in response to drought stress, UV-B radiation and in combination of drought stress and UV-B radiation. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

in response to UV irradiation was also reported for birch seedlings (*Betula verrucosa* Ehrh.) (LAVOLA et al., 1997). Also, CHAPPELL and HAHLBROCK (1984) found that UV radiation lead to the accumulation of pigments and phenolic compounds as a plant defense mechanism. It is also documented that UV-B radiation activates genes for enzymes of the phenolic compound synthesis, such as PAL and chalcone synthase (CALDWELL and BRITZ, 2006). Thus from the present results, it is concluded that quercetin and only to a certain extent luteolin might be specific UV-B protectants in lettuce.

A study by NOGUES et al. (1998) showed that UV-B radiation delayed and reduced the harmful effects of drought stress in pea (*Pisum sativum* L.) plants by reducing transpirational loss via influencing stomatal conductance and leaf area development. Furthermore, in two Mediterranean pine species (*Pinus* spp.) elevated UV-B radiation in field experiments alleviated drought symptoms similar to drought stress, such as needle loss (PETROPOULOU et al., 1995), assumed to be caused by inducing stomata closure for optimizing plant water economy. In the present study, it seems that additional UV-B mediated the effect of drought stress, thus, less luteolin was tendentiously required to be synthesized, while additional drought events on UV-B treated plants significantly inhibited quercetin accumulation. Thus, the combined treatment of drought stress and UV-B might not lead to an accumulated response on flavonoids.

Anthocyanin content

In the present study a strong increase in the total anthocyanin content in response to drought stress occurred (Fig. 7). When plants were exposed to UV-B radiation, the amount of anthocyanin were not significantly affected and increased only tendentiously. Furthermore, combined stress impact (drought + UV-B) only led to a tendentiously higher total anthocyanin content compared to the control.

CHALKER-SCOTT (2002) concluded that anthocyanins may also prevent desiccation through osmotic effects, although it was not clear, how this mechanism could explain anthocyanin accumulation. Further, drought inhibits nutrient uptake of macro- and micronutrients due to low soil water availability. Especially nitrogen deficit

detrimentally affect photosynthetic function and efficiency and lower the levels of calvin cycle enzymes (SUGIHARTO et al., 1990). This enhances the accumulation of foliar anthocyanins in leaves of many plant species (KUMAR and SHARMA, 1999). The latter finding might explain the enhanced total anthocyanin content of lettuce under drought stress conditions in this study.

Among the several flavonoid subclasses, anthocyanins are exceptional UV-B protectants. Plants enhance their total anthocyanin content as a response towards stress for photoprotecting their photosynthetic systems. Several studies demonstrated an increased anthocyanin concentration induced by UV-B treatment (HUYSKENS-KEIL et al., 2007; TREUTTER, 2010). The results of this study contradict these findings. Additionally, also SULLIVAN et al. (1996) found no change in the concentrations of UV-absorbing compounds in response to UV-B radiation. They suggested that these qualitative differences in UV-B absorbing compounds among species are due to the different adaptation mechanisms in terms of their UV-screening capability and disparity in UV-B responses.

When both stress treatments were combined, the UV-B effect on total anthocyanin content reduced only tendentiously the effect of drought stress (Fig. 7). Thus, UV-B radiation and drought might have acted synergistically and thus, increasing UV-B radiation might alleviate the effect of drought stress in lettuce plants.

Effect of drought stress and UV-B radiation on PAL activity

Flavonoids are synthesized through the phenylpropanoid pathway (DIXON and PAIVA 1995) in which three important enzymes regulate their biosynthesis: (1) phenylalanine ammonialyase transforms phenylalanine into cinnamic acid; (2) chalcone-flavanone isomerase being responsible for an early step of flavonoid biosynthesis; and (3) peroxidase(s) degrading phenolic compounds in the cell vacuole or activate precursor molecules (LIU et al., 1995).

In the present study, application of UV-B radiation and drought stress alone could not stimulate PAL activity (Fig. 8). When drought stress and UV-B were applied in combination, PAL activity was promoted in comparison to control plants. It was shown in various studies that PAL activity increased in response to stress impacts like UV and drought stress (OH et al., 2010; SHEHAB et al., 2010). However, the present results demonstrated that also enzymes other than PAL might be involved in the defense mechanism. Previous studies indicated that UV radiation can affect chalcone synthase activity or other enzymes being involved in flavonoid biosynthesis like further synthases or isomerases (CHRISTIE and JENKINS, 1996).

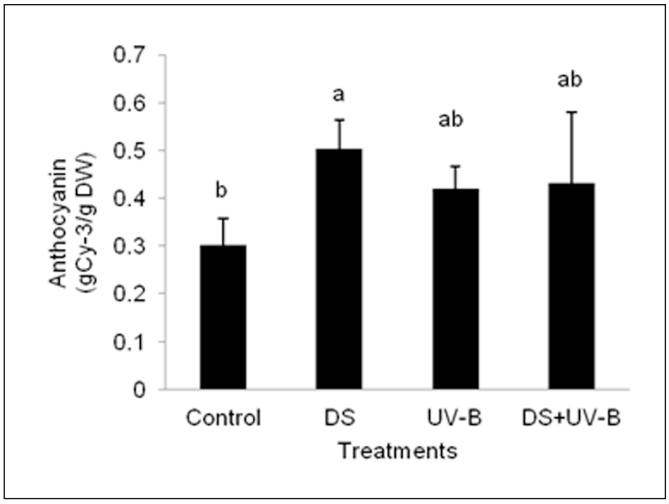

Fig. 7: Total anthocyanin content (mg Cy g^{-1} DM) of lettuce at harvest in response to drought stress, UV-B radiation and in combination of drought stress and UV-B radiation. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

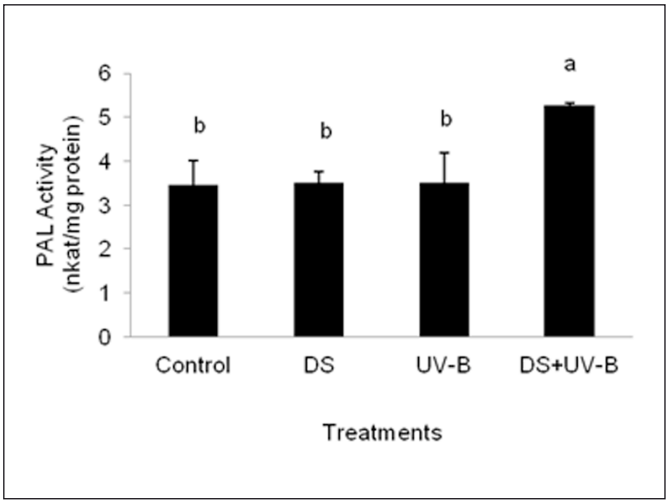

Fig. 8: Phenylalanine ammonialyase (PAL) activity (nkat mg^{-1} protein) of lettuce at harvest in response to drought stress, UV-B radiation and in combination of drought stress and UV-B radiation. Vertical bars represent standard deviation, and different letters indicate differences at the significance level $p \leq 0.05$ (Tukey test). (Control = well-watered; DS = drought stress, i.e. water-deficit; UV-B = well-watered with UV-B radiation of 0.11 kJ m^{-2}; DS+UV-B = drought stress i.e. water-deficit in combination with UV-B).

Conclusion

Plant adaptation to stress factors is associated with increased levels of antioxidant constituents that may prevent stress damage. Only to a certain extent, plants and other organisms in nature are affected by only a single stress factor. Instead, they typically respond to a combination of several factors such as UV radiation, drought stress, increased atmospheric CO_2, mineral nutrient availability, tropospheric air pollutants, and temperature. In summary, the present study revealed that lettuce plants appeared to be less sensitive to drought stress compared to UV-B in terms of biomass production and dry matter in contrast to the strong reaction regarding secondary plant metabolites. Thus, the effect of drought stress on secondary metabolites of lettuce were more pronounced than those of UV-B radiation, leading the assumption that the latter mediating drought stress effect which needs to be included in further investigations. Furthermore, the role of different flavonoids in stress adaptation of lettuce needs to be studied more in detail. However, the direction and extent of an interaction between UV-B and drought stress also depends on the physiological stage of the plant as well as on the time and duration of stress exposure which has to be considered in the future.

Acknowledgments

We would like to thank Mrs. Susanne Meier and Mrs. Sigrun Witt of the Division Urban Plant Ecophysiology for their excellent analytical support.

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